3811-Pos Board B539
TRPM3 - A Promising Target for Analgesic Treatment
Katharina Held[1], Sílvia João Pinto[1], Sara Kerselaers[1], Katrien De Clercq[1], Patrick Chaltin[2], Thomas Voets[1], Joris Vriens[1].
[1]KU Leuven, Leuven, Belgium, [2]Center for Drug Design and Development, Leuven, Belgium.
According to the American Pain Society, pain is one of the most common symptoms why patients search medical attention. A recent achievement in pain research was the identification of Transient Receptor Potential (TRP) channels as analgesic targets. The superfamily of TRP ion channels consists of 28 different members in mammalians. The sensitivity of TRP channels to a broad array of stimuli allows them to function as biological sensors involved in processes ranging from vision to taste, and tactile sensation. The so-called thermoTRPs (temperature sensitive) are typically expressed in sensory neurons, where they act as primary thermosensors for the detection of innocuous and noxious temperatures. Recently, our research group identified high TRPM3 expression in nociceptor neurons, where it plays a decisive role in the nocifensive response to pregnenolone sulphate (PS) and heat and in the development of heat hyperalgesia during inflammation.
This project aims to validate TRPM3 as a potential target for the development of new analgesics. Therefore, we purpose to identify new potent and selective TRPM3 blockers, and show their ability to cure different pain conditions in vivo.
In collaboration with the Center for Drug Design and Development (CD3) we identified a new potent TRPM3 blocker (CIM056741) that showed a high selectivity for TRPM3 compared to other related TRP channels. The blocker was able to inhibit PS induced $[Ca^{2+}]i$ signals in isolated DRG neurons. Furthermore, CIM056741 significantly reduced the sensitivity of mice and rats to noxious heat and PS-induced chemical pain. Interestingly, injection of CIM056741 strongly reduced the inflammatory induced mechanical hyperalgesia and was without effect on body core temperature, heart rate and locomotor activity.
In conclusion, the obtained results validate TRPM3 as a potential target for new analgesic treatments in humans.

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Extracellular Loops are Essential for the Assembly and Function of TRPP/PKD Complexes
Zahra Salehi-Najafabadi, Clarissa Lam, G.M. Mahmud Arif Pavel, Parul Kashyap, **Yong Yu**.
St. John's University, Queens, NY, USA.
Transient receptor potential channel polycystin subfamily (TRPP) proteins assemble with polycystic kidney disease (PKD) proteins to form functionally important complexes. For example, the TRPP2/PKD1 receptor-ion channel complex plays a critical role in renal physiology. Mutations in either protein cause autosomal dominant polycystic kidney disease (ADPKD), one of the most common genetic diseases in humans. A similar complex, assembled by TRPP3 and PKD1L3, is a candidate for the sour taste receptor. The TRPP2/PKD1 complex contains three TRPP2 subunits and one PKD1 subunit and the interaction between their C-termini is crucial for the complex assembly. The TRPP3/PKD1L3 complex has the same subunit stoichiometry but its assembly involves interactions between the transmembrane segments of both proteins. These interactions have been shown to be essential for the assembly, surface expression and function of the complexes. Here we find another novel binding site between these proteins. When co-expressed in HEK293T cells, the extracellular loops between the first and second transmembrane segments (I-II loop) of TRPP2 and TRPP3 associate with the extracellular loops between the sixth and seventh transmembrane segments (VI-VII loop) of PKD1 and PKD1L3 respectively. These loops can also associate with their binding partners when the latter is expressed as full-length proteins. The loop-loop associations are functionally crucial since expression of either the TRPP3 I-II loop or the PKD1L3 VI-VII loop shows dominate negative effect on the acid-induced current of TRPP3/PKD1L3 complex. These results demonstrate, for the first time, the previously unknown essential role that these extracellular loops play in the assembly of TRPP/PKD complexes.

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Nicotinic Acid Activates the Capsaicin Receptor TRPV1 - A Potential Mechanism for Cutaneous Flushing
Linlin Ma[1,2], Bo Hyun Lee[1], Rongrong Mao[3], Anping Cai[3], Yunfang Jia[3], Heather Clifton[1], Saul Schaefer[1], Lin Xu[3], Jie Zheng[1].
[1]University of California Davis, Daivs, CA, USA, [2]University of Queensland, Brisbane, Australia, [3]Kunming Institute of Zoology, Kunming, China.
Nicotinic acid (niacin or Vitamin B3) has been widely used in the last 50 years to treat dyslipidemias and represents an effective and safe means to reduce the risk of mortality from cardiovascular disease. Nonetheless, a substantial fraction of patients discontinue treatment due to a strong side effect of cutaneous vasodilation, commonly termed flushing. As a multi-component complex biological event, the molecular mechanism of flushing is not completely understood yet. In the present study, we tested the hypothesis that nicotinic acid causes flushing by activating the capsaicin receptor TRPV1, a polymodal cellular sensor that mediates a similar flushing response upon consumption of spicy food.
Indeed, we observed that the nicotinic acid-induced increase in blood flow was substantially reduced in $Trpv1^{-/-}$ knockout mice, indicating involvement of the channel in flushing response. Using exogenously expressed TRPV1, we confirmed that, at millimolar concentrations, nicotinic acid directly and potently activates TRPV1 from the intracellular side, but very weakly activates its homolog TRPV3 while inhibiting TRPV2 and TRPV4. Binding of nicotinic acid to TRPV1 lowers its activation threshold for heat, causing channel opening at physiological temperatures. Activation of TRPV1 by voltage or ligands (capsaicin and 2-APB) is also potentiated by nicotinic acid. Furthermore, nicotinic acid does not compete directly with capsaicin but may activate TRPV1 through the 2-APB activation pathway. Using live-cell fluorescence imaging, we observed that nicotinic acid can quickly enter the cell through a transporter-mediated pathway to activate TRPV1. In conclusion, direct activation of TRPV1 by nicotinic acid may lead to a cutaneous vasodilatory response that contributes to flushing, suggesting a potential novel pathway to inhibit flushing and improve compliance.

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Effects of TRPM7 Inhibitors on Physiological Mg^{2+} Influx in Rat Ventricular Myocytes
Michiko Tashiro, Hana Inoue, Masato Konishi.
Tokyo med.univ., Tokyo, Japan.
We measured free Mg^{2+} concentration ($[Mg^{2+}]_i$) in rat ventricular myocytes using a fluorescent indicator furaptra (mag-fura-2). $[Mg^{2+}]_i$ decreased from ~0.9 mM to 0.2-0.5 mM by incubation of the cells in a high-K^+ (Ca^{2+}- and Mg^{2+}-free) solution, and recovered by perfusion with Ca^{2+}-free Tyrode's solution containing 1 mM Mg^{2+}. The time course of the $[Mg^{2+}]_i$ recovery was fitted by a single exponential function, and the first derivative at time 0 was analyzed as an initial Mg^{2+} influx rate. In order to characterize physiological Mg^{2+} influx pathways, we used known TRPM7 inhibitors, 2-Aminoethoxydiphenyl borate (2-APB) and NS8593. The initial rate of Mg^{2+} influx was decreased to 43 ± 10 % (n=6) by 100 μM 2-APB, and to 12 ± 8.6 % (n=5) by 10 μM NS8593. These compounds inhibited the Mg^{2+} influx with half inhibitory concentrations (IC_{50}) of 17 μM (2-APB) and 2.0 μM (NS8593). 2-APB and NS8593 also inhibited Ni^{2+} influx when estimated by quenching of furaptra fluorescence with IC_{50} values of, respectively, 20 μM and 4.4 μM; these values are comparable to those for Mg^{2+} influx. Under the whole-cell patch-clamp configuration, removal of intracellular and extracellular divalent cations induced large inward and outward currents, I_{MIC}, carried by monovalent cations likely via TRPM7 channels. The I_{MIC} measured at −120 mV was diminished to 48 ± 3.6 % (n=7) by 100 μM 2-APB, and to 50 ± 12 % (n=4) by 10 μM NS8593. These results support our previous conclusion [Biophys J 102:664a, 2012] that TRPM7/MIC channels serve as a major physiological pathway of Mg^{2+} influx in rat ventricular myocytes.

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Localization and Role of Transient Receptor Potential Cation Channels in Rabbit Ventricular Myocytes
Qinghua Hu, McNary G. Thomas, **Frank B. Sachse**.
University of Utah, Salt Lake City, UT, USA.
Several members of the transient receptor potential cation (TRPC) channel family are stretch activated. They are thought to play a critical role in mechano-electrical coupling in cardiac myocytes and in cardiac hypertrophic remodeling. However, studies on their subcellular localization in cardiomyocytes are conflicting, and their functions in cardiac myocytes are barely understood. In this study, we investigated the spatial distribution of TRPC channels in isolated ventricular myocytes from adult rabbit using immunolabeling and three-dimensional confocal microscopy. Colocalization of TRPC with (i) sarcolemma labeled with wheat germ agglutinin (WGA), (ii) sarcoplasmic reticulum (SR) labeled for SR calcium ATPase (SERCA2), and (iii) cytoskeletal proteins including sarcomeric α-actinin, desmin, vinculin and β-tubulin, was assessed by a quantitative approach. Furthermore, we measured stretch-activated current with whole-cell voltage clamping. Our results from confocal imaging revealed a transverse striated distribution of TRPC1 and TRPC6 channels. We found colocalization of TRPC1 and TRPC6 with sarcomeric α-actinin, SERCA2A and desmin, but not with